

Comparison of Cord Blood pH, Haematological Parameters and APGAR Score of New-Born with Maternal and Peri-Natal Risk Factors: A Prospective Observational Study

MEENAKSHI MAHADEVAN¹, VIJAYASHREE RAGHAVAN², GEETHAPRIYA GOVINDARAJAN³, DIVYASREE PRAKASH⁴

ABSTRACT

Introduction: Appearance, Pulse, Grimace, Activity, Respiration (APGAR) score is used worldwide to assess newborn health immediately after birth. The combination of pH analysis, haematological parameters of cord blood samples and APGAR score compared with the maternal and peri-natal risk factors will help us identify the cause of low APGAR.

Aim: To compare the pH of cord blood, haematological parameters, Neutrophil-Lymphocyte Ratio (NLR) and APGAR score with high-risk maternal and peri-natal factors.

Materials and Methods: This is a prospective observational study, conducted over a period of six months, at Chettinad Hospital and Research Institute, Chennai. The cord blood samples of 40 newborns (20 normal and 20 high-risk pregnancy) were collected for assessing pH and haematological parameters (complete

haemogram, nucleated Red Blood Cells (RBCs), reticulocyte count). APGAR scores were obtained from clinical records. The data obtained in the study was analysed using the International Business Management (IBM) Statistical Package for Social Sciences (SPSS) statistics version 23.0.

Results: The authors compared 20 cases of newborns with maternal risk-factors with 20 newborns with no risk factors, and found that low APGAR and acidic pH of cord blood, high NLR was associated with risk factors present in the ante-natal and peri-natal period.

Conclusion: The present study observed that a low APGAR, low acidic pH of cord blood, high NLR was seen in mothers with adverse risk factors during pregnancy, compared to the mothers with no associated co-morbidities. Hence, the presence of risk-factors during pregnancy should warrant a close follow-up of the new-born in the immediate postpartum period.

Keywords: Appearance pulse grimace activity and respiration score, Assessment, Haemogram

INTRODUCTION

Foetal hypoxia can occur anytime during the course of pregnancy. Regular ante-natal check-up and foetal monitoring is required to assess foetal health. Electronic foetal monitoring is used to screen foetal distress during pregnancy and labour [1]. Foetal mortality continues to be high in our country despite the recent advances in maternal and foetal care. The main cause of death during the early neo-natal period is peri-natal hypoxia. Prolonged low-oxygen saturation can result in hypoxic ischaemic brain damage and in severe cases can also lead to cerebral palsy. There are several maternal and foetal risk factors that can lead to neo-natal hypoxia, some of which include intra-uterine meconium aspiration, injury during birth, certain infections [2].

APGAR score was originally developed in the mid-nineties as a tool to assess neo-natal health and as an aid for appropriate measures to be taken in case of neo-natal hypoxia. The score also helps the physician evaluate the requirement for resuscitation and chances of neo-natal survival. APGAR score is calculated at the end of one minute and five minutes. It includes five parameters: heart rate, respiration, muscle tone, reflexes, and colour. Each of these factors is given a score of 0, 1 or 2 which sums up to a total of 10 [3].

Apart from assessing the APGAR score, the pH of cord blood can also be used as an indicator of neo-natal distress. The normal umbilical cord pH is >7.2 . A pH of less than seven is considered low, and is labelled as neo-natal acidaemia and is associated with neo-natal complications [1]. Haematological parameters including RBC, White Blood Cells (WBC) and platelet counts, haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red-cell Distribution Width (RDW), Reticulocyte count and nRBCs can also be evaluated to assess neo-natal health.

The maternal and intra-natal risk factors are additionally studied, apart from neo-natal cord blood pH, haematological parameters and APGAR scores. This was done to predict neo-natal outcome by correlating these values and to exercise high caution and practice early ICU admission when there are associated risk factors along with low APGAR, cord blood pH and a high NLR.

MATERIALS AND METHODS

The present study is a prospective observational study which was conducted over a period of six months from March 2022 to August 2022 at Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India. Consent was obtained from the mothers before collecting the cord blood samples and clinical details.

Sample size calculation: Population size (for finite population correction factor or fpc)(N): 100000. Hypothesised % frequency of outcome factor in the population (p): $6.6\% \pm 5$. Confidence limits as % of 100, (absolute \pm)(d): 5% Design effect (for cluster surveys-DEFF): 1

Sample size 'n' = $\frac{DEFF * N * p(1-p)}{(d^2 / Z^2 * 1 - a/2 * (N-1) + p * (1-p))}$

Sample size = 41, as Result from OpenEpi

In the present study, the authors included 40 cases who were in their third trimester of pregnancy (37 weeks) expecting to have a normal vaginal delivery. They were separated into two groups based on the presence and absence of maternal and peri-natal risk factors. They were categorised as normal pregnancies and high-risk pregnancies. Each group contained 20 members. Advanced maternal age, maternal obesity, instrumental delivery, meconium-stained liquor, pre-gestational diabetes mellitus, prolonged rupture of membranes, pregnancy induced hypertension and urinary tract infection during pregnancy were the risk factors under consideration and those that

did not have any of these conditions were taken under the no-risk factor category.

Human ethics committee clearance was obtained (Proposal number: 508/IHEC/3-19).

Inclusion criteria: All term infants born via normal vaginal/instrument assisted delivery, irrespective of their gravid status were included in the study.

Exclusion criteria: Infants born through caesarean section, pre-term infants, still-born infants, infants for whom clinical details were not available were excluded from the study. Caesarean section cases were not included in this study.

Procedure

Immediately after the delivery of the new-born, the umbilical cord was clamped and the sample was collected in a pre-heparinised 5 mL syringe to calculate pH values using automated blood gas analyser. An Ethylene Diamine Tetra-acetic Acid (EDTA) sample was also collected to assess haematological parameters in the cord blood. The pH of the cord blood was calculated by assessing Arterial Blood Gas (ABG). The normal umbilical cord pH is 7.2 and a pH of less than seven is associated with increased incidence of cerebral palsy [1]. In this study, pH of less than seven was considered low umbilical cord pH. APGAR scores were collected from the paediatrician's register for 20 cases with risk factors and 20 cases without risk factors. APGAR stands for appearance, pulse, grimace, activity, respiration. It is scored out of a ten with each score ranging from 0 to 2.

Appearance: Normal colour overall with pink colour of the hands and feet is scored 2, normal colour but bluish hands and feet is scored 1 and bluish or pale all over is scored 0.

Pulse: Is scored 2 if it's over a 100 bpm; 1, if it is below 100 bpm and 0 if there's no pulse.

Grimace: scored 2 if the new-born pulls away, sneezes or coughs on stimulation; 1 if there is only facial movement with stimulation and 0 if there is no response to stimulation.

Activity: scored 2 if there is active movement; 1 if there is less movement and 0 if there is no movement.

Respiration: Scored 2 when there is a normal effort and good cry; 1 with slow, irregular breathing and a weak cry; 0 if there is no breathing [4].

The cord blood sample was run in our automated haematology analyser to obtain complete blood count values. In order to calculate the reticulocyte count and nucleated RBCs, blood smears were taken and stained with Leishman stain and Methylene blue were done.

History was obtained regarding the presence or absence of diabetes mellitus before pregnancy and also the values of blood sugars during pregnancy for all the mothers. Gestational diabetes mellitus was diagnosed based on a plasma glucose of >200mg/dL after a 2-hour oral glucose tolerance test [5]. The authors also procured the blood pressure values of all the expecting mothers. Pregnancy induced hypertension was diagnosed when the systolic BP was more than 140 and diastolic BP was more than 90 [6]. The age and the weight, including the Body Mass Index (BMI) were also included in the data record. Maternal age of more than or equal to 35 was considered advanced maternal age [7]. Body mass index of overweight individuals, between 25 and 29.9 was associated with increased complications during pregnancy and the same cut-off is considered in this study [8]. The mode of delivery and the presence or absence of foetal distress during delivery along with the use of any instruments was also documented. Any infection/Urinary Tract Infection (UTI) during pregnancy was taken into account. With these relevant clinical details obtained from the subjects, the authors were able to categorise them as low-risk or high-risk.

Infants requiring Neonatal Intensive Care Unit (NICU) admission and oxygen support along with the number of days in NICU were

documented. After collecting data, all the parameters of low-risk and high-risk pregnancies were compared along with the one minute and five minute APGAR scores. Total score ranges from 0 to 10, a higher score indicating the better physical condition of the baby. Scores that are lower than seven could indicate foetal distress and is associated with an increased chance of foetal demise in the early neo-natal period [3].

STATISTICAL ANALYSIS

The data obtained in the study was analysed using the IBM SPSS statistics version 23 for data analysis.

RESULTS

In the present study, the authors have 20 cases with known maternal and foetal risk-factors, which are compared with the haematological parameters (RBC, WBC and platelet counts, Hb, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red cell Distribution Width (RDW), Reticulocyte count and nRBCs), APGAR score and cord-blood pH. The other 20 cases were infants born without any associated or known risk-factors. All of them were delivered by normal vaginal delivery with or without use of instruments. The risk factors that were seen in 20 cases were, UTI during pregnancy, pregnancy induced hypertension, pre-gestational diabetes mellitus, meconium-stained liquor, prolonged rupture of membranes, advanced maternal age of >35 years, maternal obesity and instrument- assisted delivery.

The most common risk factors amongst these were: pregnancy induced hypertension, meconium-stained liquor and urinary tract infection during pregnancy [Table/Fig-1]. The parity of the patient was not considered in this study. None of the cases had any associated maternal or infant mortality.

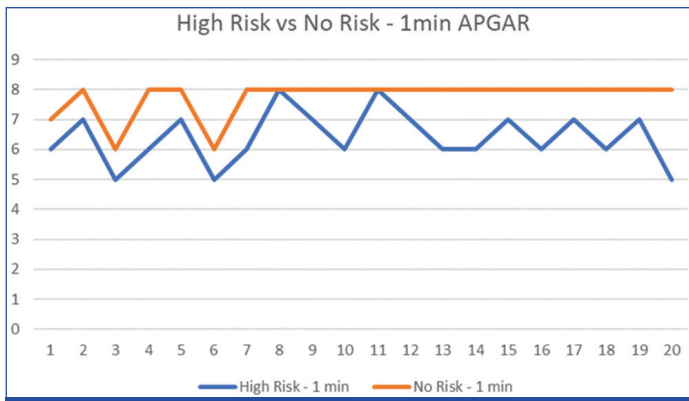
Row labels	Number of cases (N=40)	Average of pH
Advanced maternal age	2	6.60
Instrumental delivery	2	6.80
Maternal obesity	1	7.10
Meconium-stained liquor	4	6.58
Pre-gestational diabetes mellitus	1	6.70
Pregnancy induced hypertension	5	6.86
Prolonged rupture of membranes	1	6.40
UTI during pregnancy	4	6.93
Total	20	6.77

[Table/Fig-1]: High-risk factors and pH level.
UTI: Urinary tract infection

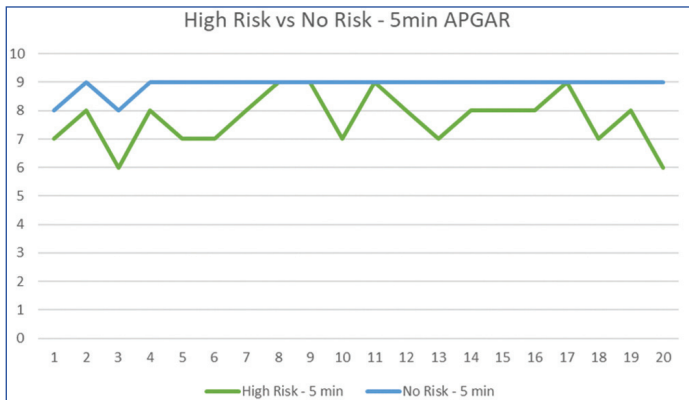
When the authors compared the one minute APGAR of pregnant females with known risk factors and with no risk factors, they found that the APGAR was normal for almost all the infants that had no associated maternal or foetal risk factors, as compared to the group that had risk factors.

Most infants with associated risk factors had a 1 minute APGAR ranging between 5/10 and 8/10 with an APGAR of 6/10 in 8 cases, 7/10 in 7 cases, 5/10 in three cases and 8/10 in two cases [Table/Fig-2]. The five minute APGAR score was also relatively low in cases with high-risk factors compared to the infants with no associated risk factors, 6/10 in two cases, 7/10 in six cases, 8/10 in eight cases and 9/10 in four cases [Table/Fig-3]. Most of the infants with no risk factors had one minute APGAR of 8/10 with only three cases having an APGAR of six and seven out of 10 [Table/Fig-2].

On comparing the five minute APGAR between high and no-risk groups, low-risk infants had a better APGAR. The five minute APGAR of infants with associated risk factors was better than the one minute APGAR. Eight cases had an APGAR of 8/10, six cases had an APGAR of 7/10, four cases had a normal APGAR of 9/10



[Table/Fig-2]: Comparison of one minute APGAR score in high-risk and low-risk infants. The one minute APGAR is lower in high-risk than in the no-risk factor group.



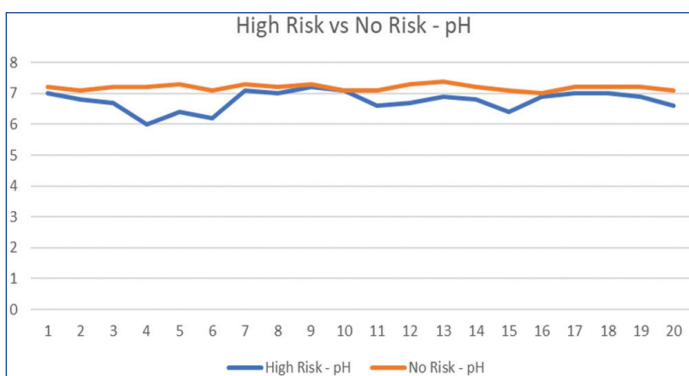
[Table/Fig-3]: Comparison of five minute APGAR score in high-risk and low-risk infants. The five minute APGAR is significantly lower in high-risk than in the no-risk factor group.

and only two cases had a persistently low APGAR of 6/10. The low/no risk group of infants had an overall higher APGAR of 9/10 and with only two cases with an APGAR of 8/10.

A pH of <7 was considered to be low in the present study. The infants with no risk factors had a higher pH of >7. These infants also had a better APGAR score. Amongst the high-risk group, the pH range was between 6 and 7.2 [Table/Fig-4]. There was no association between the risk factors and the severity of acidic pH. The cord blood pH was also measured and compared between the high-risk and low-risk infants and was found to be significantly lower (acidic) and these infants also had a low one minute and five minute APGAR [Table/Fig-5].

pH	No. of cases
≤6.4	4
≤6.8	6
≤7.2	10
Total	20

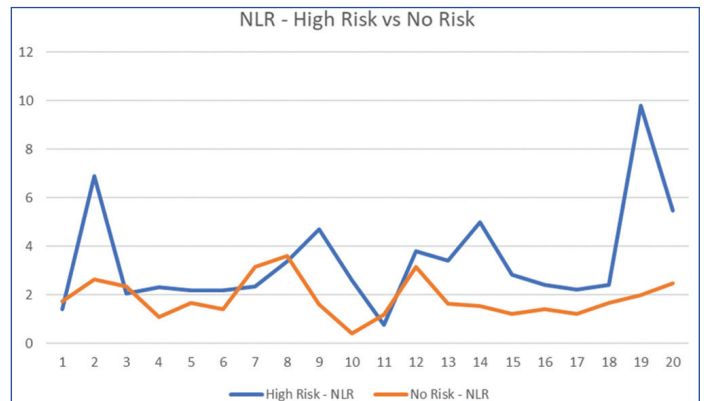
[Table/Fig-4]: Distribution of pH values amongst cases with risk factors.



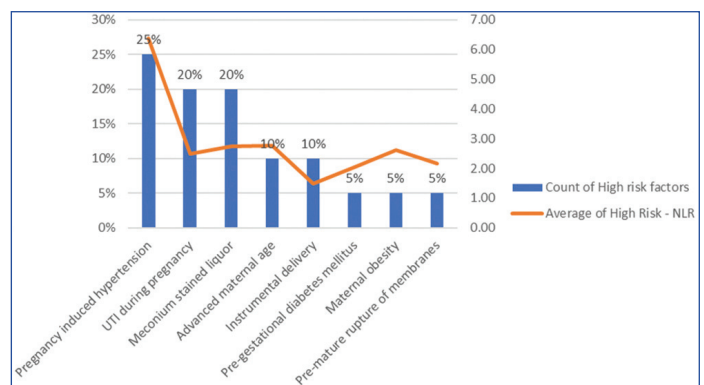
[Table/Fig-5]: Comparison of pH among high-risk and no risk infants. The pH of cord blood amongst the high-risk group was significantly lower than that of the group with no risk factors. (p=0.001)

The infants were followed-up and it was noted that 12 out of 20 infants with associated maternal and peri-natal risk factors required NICU admission and oxygen supplementation. Infants with no risk factors did not require NICU admission. None of the infants required Continued Positive Airway Pressure (CPAP) or invasive modes of ventilation. All of the 40 infants were term and none of them had any congenital abnormalities.

NLR which was found to be significantly increased in those cases with risk factors [Table/Fig-6], and a high NLR was especially seen in relation to pregnancy induced hypertension [Table/Fig-7].



[Table/Fig-6]: Comparison of NLR among high-risk and no risk group. It was observed that NLR among the infants with associated risk factors is higher than the NLR of the no-risk group infants.



[Table/Fig-7]: NLR with high-risk factors. Pregnancy induced hypertension has the maximum elevation of neutrophil-lymphocyte ratio (NLR) compared to the other risk factors.

Upon evaluating the haematological parameters, not many parameters were significant was found (p-value of each parameter summarised in [Table/Fig-8]) except for the NLR. The descriptive statistics and the Levene's test for equality of variances of all the parameters associated with and without risk-factors are tabulated in the above table [Table/Fig-8].

Parameters	High-risk		No risk		Levene's test for equality of variances	
	Mean	Standard deviation	Mean	Standard deviation	F (Upper critical value)	Significance (p-value)
Apgar-1 min	6.4	0.883	7.75	0.639	-	-
Apgar-5 min	7.7	0.923	8.9	0.308	-	-
pH	6.765	0.3183	7.19	0.0968	16.751	0.001
RBC	4.218	0.4122	4.081	0.481	0.833	0.367
nRBC	1.155	0.8476	1.46	1.7479	3.584	0.066
WBC	14.755	4.9565	13.965	6.1298	1.145	0.291
Neutrophils	66.77	11.5225	53.17	10.2698	0.682	0.414
Lymphocytes	24.205	9.894	32.12	9.2697	0.004	0.953
NLR	3.403	2.10896	1.8525	0.80817	7.057	0.011
Basophils	0.83	1.154	1.455	2.0753	3.458	0.071

Monocytes	6.27	4.2161	9.85	2.7345	2.544	0.119
Eosinophils	1.975	1.6225	3.405	2.4326	3.827	0.058
HCT	42.4	7.675	42.125	6.7721	2.368	0.132
HB	13.955	2.1977	13.64	2.1162	0.772	0.385
MCV	98.24	13.3549	103.105	10.3474	6.468	0.015
MCH	32.45	4.8255	33.405	3.3043	2.73	0.107
MCHC	33.14	3.3057	32.41	0.6973	3.019	0.09
RDW	17.33	2.5221	18.505	3.5723	0.029	0.866
Reticulocyte count	3.695	1.6488	4.92	1.7905	0.477	0.494
Platelet count	235.95	67.729	271.1	84.177	1.267	0.267

[Table/Fig-8]: Descriptive statistics of high and no risk factors and Independent samples test- p-value for all parameters.

APGAR: Appearance, Pulse, Grimace, Activity, and Respiration; RBC: Red Blood Cell; HCT: Haematocrit; Total N=20 in each group; Hb: Haemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; RDW: Red cell Distribution Width; RBC: Red Blood Cell; WBC: White Blood Cell;

DISCUSSION

In this study involving 40 participants, a clear correlation was made between low cord blood pH, low APGAR scores in association with maternal and foetal risk factors. In addition, haematological parameters were assessed in all the 40 cases to evaluate the changes related to low pH, low APGAR and risk factors. In a study conducted by Kapaya H et al., acidemia of cord blood was defined as a pH <7.2 [1]. In the present study, the cut-off pH value for an acidic pH was 7 [9]. Placenta is responsible for maintaining acid-base balance in foetal life. The capacity of the placenta to maintain appropriate pH depends on various factors, including the maternal age at the time of conception, low maternal Hb levels and other factors. The umbilical cord acidemia is attributed to such factors that influence the optimal functioning of the placenta [9].

The acidic pH of the cord blood is due to the prolonged oxygen deprivation and the degree of acidemia is based on the severity of hypoxia. In a study done by Ahmadpour-Kacho M et al., correlated neo-natal asphyxia and risk factors. The most common risk factors in their study were meconium-stained liquor, breech presentation and it was found that the APGAR score of the high-risk group was lower than that of the group with no significant risk factors [10]. In the present study, the most common risk factors were pregnancy induced hypertension, meconium-stained liquor and UTI during pregnancy.

Another study conducted by Meena P et al., included only asphyxiated infants of both normal vaginal delivery and caesarean sections whereas, in the present study, the authors made a comparison between the presence or absence of risk factors of infants born through normal vaginal delivery only. All of the infants required NICU admission after delivery in that study. They had similar results of the cord blood having an acidic pH which was associated with a low APGAR score in all the babies with birth asphyxia [11]. In the present study, although none of the infants had asphyxia, some level of hypoxia must have been present due to foetal distress caused by maternal hypertension. Severe PIH can impede the blood supply and oxygen delivery to the brain of the developing foetus. Pregnancy induced hypertension can also elevate the chances of developing meconium aspiration syndrome. Other factors such as elevated blood sugars, maternal age of more than thirty are also established risk factors for the development of meconium aspiration syndrome which can cause foetal asphyxia [12].

A study conducted by Balachandran L et al., showed that the presence recurrent of urinary tract infections was associated with pre-term delivery. However, in this study the APGAR score of infants or cord blood pH values were not correlated [13]. In a study conducted by Kapaya H et al., found strong association between UTI and acidemia of cord blood. Irrespective of the frequency, even if one episode of UTI can lower umbilical cord pH. They also found

that the pH was much lower in emergency caesarean sections rather in elective Lower Section Caesarean Section (LSCS) or normal vaginal delivery, which is attributed to the increased period of foetal distress which brings about the decision of converting a vaginal delivery to a caesarean section [1].

In the present study, long-term follow-up of infants with low APGAR and low cord blood pH was not done and none of the infants developed Hypoxic Ischemic Encephalopathy (HIE). In a study conducted by Malin GL et al., only 10% of the babies with HIE, which is a consequence of low pH of arterial cord blood, developed cerebral palsy later in life. The strength of association between HIE and cerebral palsy is controversial due to the presence of other factors such as low-birth weight, seizures etc., [14].

Although diabetes is very common in pregnancy, in this study, only one case has pre-gestational diabetes and had a very low APGAR and a pH of only 6.7. Gestational diabetes and APGAR had no correlation with each other, according to a study done by Yeagle KP et al., since only one case had pre-gestational diabetes in the present study, no correlation can be made between APGAR score and diabetes mellitus [5].

Based on the results obtained, some of the risk-factors such as PIH, UTI, advanced maternal age, increased BMI were associated with foetal hypoxia that was evident with cord blood acidemia, and a low APGAR score. Although none of the infants suffered severe hypoxic injury, some degree of hypoxia was present and with appropriate management with oxygen and close monitoring, there was no mortality associated with any of the cases. However, in this study, long term follow-up in order to assess the developmental milestones and the general well-being of the child was not done.

NLR has been identified as one of the markers of inflammation which has been found to be elevated in cases of chronic inflammation, like coronary artery disease, renal abnormalities etc. It was also found to be a prognostic marker in terms of predicting severity of any inflammatory condition. In a study done by Okoye HC et al., NLR was found to be high in cases with pregnancy induced hypertension, which is concordant with the present study [15]. All the cases with PIH in this study were associated with a low APGAR and cord blood acidemia as well.

The significance of this parameter is still pending for further analysis by future studies. NLR had no correlation with the other risk factors elaborated in the present study. Other haematological parameters such as RBC, platelet counts, MCV, MCH, MCHC, RDW, Reticulocyte count and nRBCs had no correlation with any of the parameters under investigation in this study.

Limitation(s)

Long-term follow-up of the infants was not possible and hence were not included in this study.

CONCLUSION(S)

Assessing NLR with cord blood pH and APGAR scores could prove to be of utility in women with high-risk pregnancies and problematic deliveries, and can be added as routine investigations in post-natal assessment. These parameters can serve as combined or individual factors to assess foetal health in the immediate post-partum period, especially in the presence of risk factors.

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PARTICULARS OF CONTRIBUTORS:

1. Postgraduate, Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India.
2. Professor, Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India.
3. Assistant Professor, Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India.
4. Student, Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Geethapriya Govindarajan,
Assistant Professor, Department of Pathology, Chettinad Hospital and Research Institute, Kanchipuram District, Rajiv Gandhi Salai, Kelambakkam, Chennai-603103, Tamil Nadu, India.
E-mail: geethgovindarajan@gmail.com

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